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COMPLETE SPECIFICATION

NO DRAWINGS

Diagnostic Agent

We, CHAS. PFIZER & CO., INC., a Corporation organized under the laws of the State of Delaware, United States of America, of 235 East 42nd Street, New York, State of New York, United States of America, do hereby declare the invention, for which we pray that a Patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a diagnostic agent for syphilis. In particular, it is concerned with a proficient and rapid syphilitic antigen agglutination test.

Syphilis is a disease which has afflicted humans for centuries. In recent years, this infectious disease has been effectively controlled and cured by penicillin therapy. This chemotherapeutic cure has been greatly aided by analytical methods which have been developed to diagnose this infectious condition. However, most of the presently available serological tests for syphilis are time consuming, as well as complicated, even when conducted by experienced technicians. The improved diagnostic test of the present invention is both an accurate and rapid method of detecting the aforesaid affliction.

The present invention concerns an improvement of a prior diagnostic test for syphilis which was published by Charles T. Uyeda in the "Technical Bulletin of the registry of Medical Technologists" volume 33, No. 8, pages 139-143 (1963). The advantage of this improved procedure is that the test serums are rapidly and effectively inactivated by contact with from 4 to 6% solution of choline chloride, while the prior art procedure requires inactivation of said serum by heating for approximately 30 minutes.

The diagnostic test of this invention is based upon the known interaction of syphilis antigen with its corresponding antibody. When contacted under the proper conditions, they interact to form a complex which is less soluble than either of the uncombined original components. To increase the discernibility of this action, a carrier is employed to expedite the macroscopic visualization of the reaction. In accordance with the invention the carrier employed comprises polystyrene latex particles having a particle size range from 0.3 to 2.0 μ.

The diagnostic agent of the invention comprises an aqueous suspension containing 0.1-3%, preferably 0.5-1.5% w/v of insoluble antigen-coated polystyrene particles wherein the antigen is derived from an ethanolic solution containing approximately 0.03% cardiolipin, 0.9% cholesterol and 0.2% lecithin and having a particle size from 0.3-2.0 μ, and 4-6% w/v of choline chloride.

In general, the diagnostic agent of this invention may be made by admixing 19 parts by volume of from about a 0.03 to a 0.06% w/v suspension of polystyrene latex, suspended in a saline solution, such as Kolmer's saline solution which contains 0.85% sodium chloride NaCl and 0.01% $MgSO_4$ in distilled water with approximately 1 part by volume of an antigen ethanolic solution containing approximately 0.03% cardiolipin, 0.9% cholesterol and 0.2% lecithin. This admixture is achieved by the drop-wise addition of the latter solution to the former suspension with continuous stirring until a uniform suspension is formed. This suspension of insoluble cardiolipin coated particles is then concentrated by centrifugation and subsequent decantation of all the supernatant liquid. Thereafter, the residue comprising the insoluble cardiolipin coated particles is resuspended with a saline solution containing from 4 to 6% choline chlo-

ride to a volume which will produce a suspension having an insoluble cardiolipin coated particle concentration of from 0.1-3%, preferably 0.5%—1.5% w/v. Preferably the aqueous suspension consists of 1.0% w/v of antigen-coated polystyrene particles having a particle size of 0.8 μ, and 5% w/v of choline chloride.

In use, one part by volume of said suspension is mixed with from about 2 parts by volume of the test blood serum. This latter admixture may be and is preferably, conducted with minimum quantities on a glass slide. A positive syphilitic serum is characterized by the immediate visual appearance of agglutinated particles. Alternatively, a negative serum is characterized by the absence of said agglutination and the mixture remains milky and opalescent.

It has been noted that by serial dilution of syphilitic blood serum which is admixed with the suspension of cardiolipin coated particles, that a semi-quantitative determination of the syphilitic infection is achieved by comparing the amount of agglutination produced thereby with a known standard. Further, it has been observed that the greater the syphilitic infection, the faster the agglutination time.

The following examples are given by way of illustration.

EXAMPLE I

To 9.5 ml. of Kolmer saline solution in an acid cleaned screw cap tube, there is added with stirring 0.1 ml. of 5.0% aqueous suspension of polystyrene latex particles having a 0.791μ diameter. To the resulting suspension, there is added drop by drop, with agitation, 0.5 ml. of 95% ethanol containing 0.015 mg. (0.03%) cardiolipin, 0.45 mg. (0.9%) cholesterol and 0.11 mg. (0.22%) lecithin. The resulting suspension is centrifuged for 15 minutes at 1500 RPM and the supernatant liquid is decanted and discarded. The residue is reconstituted in 0.5 ml. of Kolmer's saline solution containing 5% choline chloride. To a glass slide containing two minums of syphilitic human blood serum, there is added with stirring one minum of the aforesaid suspension. A macroscopic agglutination of the particles immediately developed indicating a positive reaction. When the aforesaid procedure was repeated with non-syphilitic human blood serum, the mixture remained milky and opalescent with no agglutination occurring.

EXAMPLE II

To 19.0 ml. of Kolmer saline solution in an acid cleaned screw cap tube, there is added with stirring 0.2 ml. of 6.0% aqueous suspension of polystyrene latex particles having a 0.557μ diameter. To the resulting suspension, there is added, drop by drop with agitation, 1.0 ml. of an ethanolic solution containing 0.03 mg. (0.03%) cardiolipin, 0.9 mg. (0.9%) cholesterol and 0.21 mg. (0.21%) lecithin. The resulting suspension is centrifuged for 20 minutes at 1500 RPM and the supernatant liquid decanted and discarded. The residue is reconstituted in 1.0 ml. of Kolmer's saline solution containing 4% choline chloride. To a glass slide containing four minums of syphilitic human blood serum, there is added with stirring two minums of the aforesaid suspension. A positive reaction was indicated by the immediate development of a macroscopic agglutination.

The aforesaid procedure was successfully repeated employing a polystyrene latex suspension containing latex particles having a 0.301μ diameter.

EXAMPLE III

To 9.5 ml. of Kolmer saline solution in an acid cleaned screw cap tube, there is added with stirring 0.1 ml. of 3.0% aqueous suspension of polystyrene latex particles having a 1.305μ diameter. To the resulting suspension, there is added drop by drop by agitation, 0.5 ml. of 95% ethanol containing 0.015 mg. (0.03%) cardiolipin, 0.45 mg. (0.9%) cholesterol and 0.11 mg. (0.21%) lecithin. The resulting suspension is centrifuged for 15 minutes at 1500 RPM and the supernatant liquid is decanted and discarded. The residue is reconstituted in 0.5 ml. of Kolmer's saline solution containing 6% choline chloride. To a glass slide containing four minums of syphilitic human blood serum, there is added with stirring, one minum of the aforesaid suspension. A macroscopic agglutination of the particles immediately developed indicating a positive reaction. When the above procedure was repeated with a non-syphilitic blood sample, no agglutination occurred.

The aforesaid procedure was successfully repeated employing a polystyrene latex suspension containing latex particles having a 2.00μ diameter.

WHAT WE CLAIM IS:—

1. An aqueous suspension containing 0.1-3%, preferably 0.5-1.5% w/v of insoluble antigen-coated polystyrene particles wherein the antigen is derived from an ethanolic solution containing approximately 0.03% cardiolipin, 0.9% cholesterol and 0.2% lecithin and having a particle size from 0.3-2.0μ, and 4-6% w/v of choline chloride.

2. An aqueous suspension consisting of 1.0% w/v of insoluble cardiolipin coated polystyrene particles having a particle size of 0.8μ, and 5% w/v of choline chloride.

3. The aqueous suspension substantially as hereinbefore described and as set forth in the Examples.

STEVENS, LANGNER, PARRY & ROLLINSON,
Chartered Patent Agents,
Agents for the Applicants.

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